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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	John C. Reed	Confirmation No.:	7441
Serial No.:	09/724,425	Art Unit:	1635
Filed:	November 28, 2000	Examiner:	Schmidt, M.
For:	**Regulation of BCL-2 Gene Expression**	Attorney Docket No:	10412-026-999

DECLARATION OF FINBARR E. COTTER UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
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Sir:

I, FINBARR E. COTTER, do declare that:

1. I am a citizen of the United Kingdom, and am Professor of Experimental Haematology at Barts and the London School of Medicine, located at Turner Street, London El 2AD, United Kingdom. I have extensive experience in the field of antisense technology, particularly in the use of antisense as a therapeutic.

2. I have been asked to comment on the teachings of the above-identified patent application (the "Reed application"), the disclosure of which is identical to PCT WO 95/08350, (the "Reed PCT") and what these teachings would provide to one skilled in the art as of the 1988 priority date of the Reed application. Additionally, I have been asked to comment on the applicability of the teachings of the Reed application and the in vitro and cell-based data presented therein for the development of bcl-2 directed antisense oligonucleotides ("AO") as therapeutic agents for the treatment of bcl-2 related disorders.

3. The Reed application relates to AO that target the bcl-2 gene and the use of such AO for the treatment of disorders relating to over expression of the bcl-2 gene product, such as cancer and autoimmune disease (*see e.g.*, the Reed application and the

Reed PCT at page 3, lines 1-16 and at page 17, lines 7-17). The Reed application describes AO that specifically target and hybridize to strategic sites of the bcl-2 gene (SEQ ID No. 19), including the translation initiation sequence, the splice donor or splice acceptor sequence of SEQ ID No.19, at least one of the first six codons of the open reading frame of SEQ ID No. 19, or the 5'- cap region of SEQ ID No. 19 (*see e.g.*, the Reed application and the Reed PCT at page 3, lines 17-24; page 4, line 1 to page 5, line 4; page 10, lines 3-6; page 12, lines 17-29). The Reed application demonstrates the ability of these AO to specifically target and hybridize to the bcl-2 gene (SEQ ID No. 19) (*see e.g.*, the Reed application at page 12, lines 6-7; *see* the Reed PCT at page 12, lines 8-9). The Reed application also provides cell-based data which demonstrates the ability of these AO to specifically target and hybridize to the bcl-2 mRNA in its native form and effectively result in cell death (s*ee,* Example 3 in the Reed application and the Reed PCT at page 22, line 9 to page 24, line 4). These cell-based data demonstrate the ability of the AO of the Reed application to permeate the cell membrane of a eukaryotic cell and have a specific antisense effect, while demonstrating that control sense oligomers completely lack the ability to affect cell growth. The Reed application further discloses the administration of such OA, either alone or in combination with antineoplastic agents, to patients in order to treat cancer and other neoplastic diseases, as well as for the treatment of autoimmunes disease. (*see e.g.,* the Reed application at pages 14-17)

4. The fundamental concerns in developing an AO as a therapeutic agent have been the appropriate design of an AO to effectively target an accessible site within the target RNA that results in a sequence-specific down regulation of the target gene product (*see e.g.,* Cotter, 2000, *Antisense Therapy for Malignancy*, in *2000 Education Book, American Society of Clinical Oncology Annual Meeting,* 338-348 at 339, col. 2, first paragraph "[I]t is becoming clear that an AO that shows specific binding to the target mRNA, that cleaves it in the presence of RNAseH, and that causes a reduction is effective for therapy."; Kuss, 1999 *Antisense-time to shoot the messenger.* Annals of Oncology, 495-503 at 497, Col. 1, para. 4. "It is becoming clear that an effective antisense molecule, rigorously evaluated to demonstrate an antisense specific effect against the target gene, will be therapeutically useful. However it is essential to target the correct gene of interest, to obtain the full potential."). The Reed application addresses these concerns and provides examples of bcl-2 AO that successfully hybridize to strategic sites within the

target RNA in its native state, resulting in sequence-specific down regulation of the resulting gene product. Over expression of the bcl-2 gene product has been implicated in disorders such as cancer and autoimmune disease. Thus, the bcl-2 AO described in the Reed application are examples of effective antisense molecules that may be used as therapeutic agents for the treatment of cancer and autoimmune disease. In fact, one of the AO of the Reed application (SEQ ID No. 17) is currently in Phase III clinical trials for treatment of non-Hodgkin's lymphoma, leukemia, prostate cancer, and melanoma, having demonstrated antitumor activity in Phase II clinical trials (G3139; Genta , Inc.).

5. The fundamental concepts for the development of bcl-2 AO for use as therapeutic agents include: (1) *Design of a specific bcl-2 AO that targets an accessible site of the bcl-2 gene*. The Reed application teaches one of skill in the art that to design an *effective* bcl-2 AO, one needs to specifically target *strategic accessible sites* of the target bcl-2 mRNA. The Reed application provides examples of such strategic sites, *e.g.*, translation initiation site, splice donor or acceptor site, or the first six codons of the bcl-2 gene (s*ee*, *e.g.*, the Reed application and the Reed PCT at page 3, lines 17-24; page 4, line 1 to page 5, line 4; page 10, lines 3-6; page 12, lines 17-29). (2) *Sequence specificity of bcl-2 AO*. According to the teachings of the Reed application an *effective* bcl-2 AO targets the bcl-2 gene in a *sequence-specific manner*. All the working examples presented in the Reed application demonstrate the bcl-2 AO antisense effect relative to control oligomers and show that the decrease in bcl-2 protein production is sequence-specific as it occurs with the AO of the Reed application, but does not occur with the sense control oligonucleotides (*see, e.g.*, Example 3 in the Reed application and the Reed PCT at page 22, line 9 to page 24, line 4). The success of the antisense technology, with respect to bcl-2, as a therapeutic agent where others have failed can thus clearly be attributed to the teachings of the Reed application.

6. The efficacy of the bcl-2 AO set forth in the Reed application is demonstrated using both *in vitro* and cell-based assays. The Reed application provides experimental evidence of the ability of the bcl-2 AO to specifically target strategic sites of the bcl-2 gene in a sequence-specific manner (s*ee,* Examples 2-18 in the Reed application and the Reed PCT at page 21, line 16 to page 57, line 19). The data presented unequivocally shows that bcl-2 AO, including G3139 (SEQ ID No. 17), as designed in

accordance with the teachings of the Reed application provide specific inhibition of lymphoid and leukemic cell growth by reducing bcl-2 gene expression (s*ee,* Example 3 in the Reed application and the Reed PCT at page 22, line 10 to page 24, line 4). Additionally, the Reed application demonstrates that the bcl-2 AO of the invention, including G3139 (SEQ ID No. 17), mediate programmed cell death (s*ee,* Example 12 in the Reed application and the Reed PCT at page 33, line 3 to page 35, line 7) and increase the chemosensitivity of neoplastic cells to chemotherapeutic agents (*see,* Example 18 in the Reed application and the Reed PCT at page 42, line 5 to page 57, line 11). In fact, the *in vitro* and cell-based assays of the Reed application convincingly show that *specific* binding of bcl-2 AO to the target bcl-2 gene reduces bcl-2 gene expression and results in the death of those cells that over express bcl-2. Once this has been achieved, preclinical development, including *in vivo* animals studies, and then clinical development with clinical trials in the disease of interest, such as malignant disease, e.g., lymphoma, is a matter of routine experimentation.

7. There have been a number of false starts with AO based cancer therapy due, in part, to certain limitations, such as specificities of the AO's, susceptibility to degradation, limited cellular bioavailability, and non-specific effects (Cotter, 2000, "Antisense Therapy for Malignancy", 338-348). However, each of these challenges have been overcome by the bcl-2 AO described in the Reed application, as demonstrated by the cell-based data provided therein. The AO presented in the Reed application have a specific antisense effect as confirmed by the complete inability of the sense control oligonucleotides to affect cell growth (s*ee e.g.,* Example 3 in the Reed application and the Reed PCT at page 22, line 10 to page 24, line 4). The AO presented in the Reed application are able to permeate the cell membrane and effectively target the bcl-2 mRNA in its native state, thus demonstrating their cellular bioavailability (see *e.g.*, Examples 3, 12, and 18 in the Reed application and the Reed PCT at page 22, line 10 to page 24, line 4; page 33, line 3 to page 35, line 7; and at page 42, line 5 to page 57, line 11, respectively). Further, the limited susceptibility of these AO to degradation is demonstrated by the delivery of these AO to cell culture medium, and their ability to traverse the cell membrane intact and successfully target the bcl-2 mRNA (see *e.g.*, Examples 3, 12, and 18 in the Reed application and the Reed PCT at page 22, line 10 to page 24, line 4; page 33, line 3 to page 35, line 7; and at page 42, line 5 to page 57, line

11, respectively). The data presented in the Reed application clearly provided the information needed by one of ordinary skill in the art to overcome the obstacles facing AO based bcl-2 therapeutics as of the 1988 priority date of the Reed application.

8. While the Reed application does not provide data from animal based studies demonstrating the efficacy of the bcl-2 AO, using routine experimentation, one skilled in the art, as of the 1988 priority date, could simply apply the teachings of the Reed application related to the *in vitro* and cell-based data presented in the Reed application, to obtain animal model data. One skilled in the art could use methodology routinely used as of the 1988 priority date, for example, an animal model, such as a nude mouse xenograft model, to confirm the efficacy of the bcl-2 AO of the Reed application and also could assay the pharmacokinetic and toxicity profiles of the bcl-2 AO. (*see, e.g.*, Yang, *Tumor Regression of Human Breast Carcinomas By Combination Therapy of Anti-Bcl-2 Antisense Oligonucleotide And Chemotherapeutic Drugs.* Proc AACR, Vol. 40, Abstract 4814, 1999, reporting the efficacy, in a nude mouse model, of one of the bcl-2 AO of the Reed application, in combination with chemotherapeutic agents, as taught by the Reed application.) The use of these methods to determine appropriate formulations and dosages of the bcl-2 AO as a therapeutic modality is a matter of routine experimentation involving phase I, II, and III clinical trials. While such experimentation may be time-consuming and arduous, it is routine and would not be considered undue as of the priority date of the Reed application.

9. In fact, the teachings of the Reed application have been successfully applied in more than one type of animal model in order to obtain animal data using at least one of the bcl-2 AO of the Reed application (G3139; SEQ ID No. 17) (Cotter *et al.*, 1994, *Oncogene*, 9(10): 3049-55). In a non nude-mouse model, cells derived from a patient with a B-cell lymphoma bearing the t(14;18) chromosomal translocation were injected into SCID mice. SCID/SCID immunodeficient mice were chosen because they have been demonstrated to readily engraft with human acute lymphoid leukemias (also of the B-cell lineage) and give a similar distribution of disease as the human counterpart when inoculated intravenously (Bosma *et al.*, 1983, *Nature*, 301: 527-530; McCune *et al.*, 1988, *Science*, 241: 1632-1639). Having established a lymphoma model, the cells were treated with bcl-2 AO. The antisense treated mice failed to develop lymphoma compared

to the sense, nonsense, and untreated mice. As demonstrated in a subsequent study, of sixty mice treated with a bcl-2 AO (G3139, SEQ ID No. 17 of the Reed application), 83% showed almost complete ablation of the lymphoma; and in fact extension of the treatment to three weeks, showed complete eradication of the lymphoma in all animals, even at the PCR level (Cotter *et al.*, 1996, *Ann. Oncol.* 7 (Suppl. 3): 32). These studies clearly demonstrate the efficacy of SEQ ID No. 17 as a therapeutic. Indeed, as demonstrated by the *in vitro* and cell-based assays presented in the Reed application all of the bcl-2 AO described show the same specific binding to the bcl-2 gene and reduction in bcl-2 gene expression as demonstrated for SEQ ID No. 17. Therefore, all of the bcl-2 AO described in the Reed application could be analogously tested for effectiveness in a clinical setting as demonstrated by the clinical utility of G3139 (SEQ ID No. 17), using routine experimentation. Indeed, as I have previously noted, even though only G3139 of the AO of the Reed application, has been taken into clinical development, other AO of the Reed application could also be taken in clinical development by one of ordinary skill in the art. (*See,* Cotter, *Human Bcl-2 Antisense Therapy For Lymphoma,* Biochemica et Biophysica Acta, 1489, 1999, 97-106, at 98, "Whatever the mechanism, the important consideration in assessing antisense effect is to establish sequence specificity of the AO against control oligomers, and to demonstrate a decrease in the amount of protein produced by the gene targeted. This has been demonstrated for G3139 and G3854 using both sense and nonsense controls.") Thus, a scientist working in the field of antisense technology, provided with the Reed application, would have the benefit of the *in vitro* and cell-based data provided therein, and could then, as a matter of routine experimentation, test the efficacy of the bcl-2 AO in an animal model using routine assays to determine pharmacokinetic and toxicity profiles, in order to formulate the bcl-2 AO as a therapeutic, and then proceed with the clinical development of such AO.

10. During the 1980s, the same time period to which the Reed application claims priority, a host of targets have led to AO currently in clinical trials for treatment of malignancies which is further proof that it was within ordinary skill in the art to overcome the challenges that faced the researcher in the field of AO based therapies (Cotter, 2000, "Antisense Therapy for Malignancy", 338-348; Kuss B. & F. Cotter, 1999, *Annals of Oncology*, 10: 495-503; Cotter *et al.*, 1999 *Biochimica et Biophysica Acta* 1489:97-106). These targets include, for example, bcl-2 (G3139, Genta Inc.), PKA (GEM®231,

Hybridon Inc.), c-myc (NeuGene®, AVI Biopharma Inc), and c-myb (Lynx Therapeutics, Inc.). Almost all the AO currently in clinical trials (including G3139, which is one of the AO of the Reed application; SEQ ID No. 17) were designed in accordance with the methodology set forth in the Reed application for the development of bcl-2 AO, *i.e.*, they were designed to target strategic accessible sites in a sequence-specific manner. Additionally, comprehensive and fundamental *in vitro* and cell-based analysis of the AO laid the groundwork for developing them as therapeutic agents. In fact, the rate limiting step and challenge in the design and development of AO has been the appropriate design of the AO's and the establishment of the specificities of the AO's an *in vitro* system which translate to a cell-based system. It was the nature of these challenges and hardships that faced the researcher that contributed to much of the unfortunate skepticism surrounding the field of antisense technology. However, once these challenges have been overcome, the AO have advanced to a clinical setting as lead compounds in cancer therapeutics (B. Kuss & F. Cotter, 1999, *Annals of Oncology* 10: 495-503).

11. The development of NeuGene® (NeuGene® AVI Biopharma, Inc.) is another example analogous to the development of G3139 (SEQ ID No. 17 of the Reed application, Genta Inc.), whereby *in vitro* and cell-based assays in the late 80's were the basis for the development of the compound as a lead drug in human clinical trials. NeuGene® antisense technology is designed to treat diseases involving abnormal cell division, such as cancer and certain cardiovascular and inflammatory diseases, including restenosis. The NeuGene® target is the c-myc proto-oncogene, which has been implicated in tumorigenesis and proliferation of smooth muscle cells, as evidenced for example, by proliferating cells that have higher levels of c-myc mRNA. Studies by Wickstrom and colleagues showed that c-myc AO inhibit c-myc protein expression and cell proliferation in a sequence-dependent and dose-dependent manner in a human promeylocytic leukemia cell line HL-60 (Wickstrom *et al.*, 1988, *Proc. Natl. Acad. Sci. U.S.A.*, 85(4): 1028-32). These results were in agreement with a number of other cell-based assays utilizing normal human T lymphocytes (Heikkila *et al.*, 1987, *Nature*, 328: 445-449); human arterial smooth muscle cells (Bedecked *et al.*, 1992, *Basic Res. Car idol.* 87(6): 585-91; Shi *et al.*, 1993, *Circulation*, 88(3): 1190-5); and human prostate cancer lines (Balladic *et al.*, 1997, *Urology*, 50(6): 1007-15). Subsequent to the establishment of the efficacy of c-myc AO in cell-based model systems, the c-myc AO

were employed in clinical settings (Kipshidze *et al.*, 2001, *Curr. Opin. Mol. Ther.* 3(3): 265-77), which lead to the eventual marketing of the c-myc AO as the NeuGene® product.

12. In summary, the Reed application provides instructions for the development and design of bcl-2 AO and shows the efficacy of such molecules in *in vitro* and in cell-based assays, despite the experimental challenges. While the Reed application does not provide data in an animal model, it teaches the use of bcl-2 AO for the treatment of disease, including neoplastic disease, either alone or in combination with other anti-cancer agents. It would be routine experimentation for one skilled in the art as of the 1988 priority date to determine the efficacy, in an animal model, of the bcl-2 AO described in the Reed application, and then to develop the bcl-2 AO for therapeutic use. Once the efficacy of the bcl-2 AO had been confirmed using an animal model, one skilled in the art readily could assay the pharmacokinetic and toxicity profiles of the bcl-2 AO, determine appropriate formulations and dosages to utilize the bcl-2 AO as a therapeutic, and then proceed with the routine clinical trials necessary to demonstrate efficacy in order to develop a therapeutic product. While such experimentation may be time-consuming and arduous, it would not be undue as of the priority date of the Reed application. Therefore, one skilled in the art would be able to develop an effective bcl-2 AO therapeutic based on the teachings of the Reed application and the *in vitro* and cell-based data provided therein.

Date: May 19, 2003